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INTERNATIONAL, A.I.E.
03006 Alicante (ES)**(54) METHOD FOR OBTAINING APOLAR AND POLAR EXTRACTS OF CURCUMA AND APPLICATIONS THEREOF**

(57) The process for obtaining the apolar extract comprises: (a) extracting the rhizomes with an organic solvent; (b) filtration and evaporation to dryness of the extract; (c) dissolution of the oleoresin obtained in hot conditions, precipitation while allowing to cool down and filtration of the solid; (d) drying and recrystallizing the solid in order to obtain a product having a purity in curcuminoids higher than 90 %. Obtaining the polar extract comprises: (a') extraction of the rhizomes with water at 50-70 °C and (b') filtration and evaporation of the water. Application to the fabrication of compositions and preparations useful as catchers of free radicals and antiaging agents, as well as reducing agents to reduce the plasma levels of lipid peroxides in human beings.

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Description**TECHNICAL FIELD OF THE INVENTION**

5 The present invention fits in the technical field of the obtainment of plant extracts with different medicinal, cosmetic, dietetic applications, etc.

More especially, the present invention provides a method for obtaining apolar and polar extracts of Curcuma that have important applications as catchers of free radicals and antiageing agents.

10 PRIORITY ART OF THE INVENTION

Curcumin and other curcuminoids, such as the essential oils obtained from plant extracts of the Curcuma and in general of the Zingiberaceae family, are useful for the treatment and prophylaxis of different diseases. For example, one can cite: EP568,001 (antiviral agents), EP440,885 (antiinflammatory agents), EP319,058 (against loss of hair), EP256,353 (treatment of malabsorption syndromes), US 5.108.750 US4,906,471, US4,842,859 platelet antiaggregation agents and anticholesterol agents); WO88/05304 (against hepatitis B and AIDS), WO88/08713 (treatment of neurological disorders), US4,568,546 (antivenin agent). The antioxidantizing and dyeing properties of curcumin and of the derivatives thereof are also known, thus, they are widely used in the food and cosmetic industries as natural preservatives (US5,266,344, KK KOBE SEIKO SHO).

On the other hand, it is known that the excess of reactive oxygen and free radicals in tissues produces alterations that can lead to premature ageing of cells and the onset of different diseases. Giving the growing concern for this phenomenon, it is not surprising that there are numerous documents about the preparation of plant extracts with antioxidantizing effects, catchers of free radicals and therefore useful in the prevention of ageing of cells. However, in the specific case of Curcuma longa extracts the number of documents found is not very large and for the most part they are not patent literature.

Among the bibliographic references of the most interesting patents existing in the data banks, application FR2,655,054 (PACIFIC CHEMICAL) about the cellular protection agents contained in curcuminoids (curcumin, 4-hydroxycinnamoyl (pheruloly)methane, bis (-hydroxycinnamoyl) methane, etc.) obtained from Curcuma longa, ascorbic acid and/or dismutase superoxide (DSO) stands out. Curcuminoids have a known antioxidantizing effect, as well as ascorbic acid and DSO that carry out a synergic effect.

On the other hand, the antiinflammatory activity of curcuminoids can also result from their antioxidantizing activity. In this sense, application EP550,807 (STEIGERWALD ARZNEIMITTELWERK) describes Curcuma longa preparations useful for the treatment of inflammatory diseases associated with the excess production of leukotrienes and prostaglandins (Crohn's disease, bronchial asthma, psoriasis, etc.) According to this document, curcumin carries out its antiinflammatory action on the one hand participating in the reactions of elimination of active oxygen and free radicals and on the other hand by inhibiting the cyclooxygenase and lipoxygenase, enzymes responsible for the synthesis of prostaglandins and leukotrienes respectively.

In connection with the methods of extraction of the polar and apolar fractions of the Curcuma longa rhizomes, they are conventional methods that use usual solvents (liquids in normal conditions) or else liquid CO₂ or NO₂ or in supercritical conditions, which is likewise usual for the extraction of fragrances and essential from plants "Carbon dioxide extracted ingredients for fragrances", Flavours & Fragrances O-5M385P, North Albert Road, Reigate, Surrey RH2 9ER, England.)

However, it is a field of noteworthy interest in which it is still desirable to obtain pure extracts, to discover possible new applications of the same in the medical-pharmaceutical fields, in cosmetics, dietetics, etc.

Along these lines, the present invention provides apolar and polar extracts of Curcuma longa with important properties as catchers of free radicals and, consequently, antiageing agents of cells. Likewise, these extracts have shown the capacity to reduce plasma levels of lipid peroxides in humans.

On the other hand, according to the bibliographic data it is known that oxygenated radicals generated in cells are controlled by the cell defense system. The excess generation of such radicals can exceed the protective capacity of the defense system and lead to oxidation of the cell components, proteins, lipids and DNA. These oxidative processes are relevant in the pathogenesis of diseases such as arteriosclerosis and rheumatoid arthritis.

DETAILED DESCRIPTION OF THE INVENTION

Just as it is indicated in the title, the present invention refers to a method for obtaining apolar and polar extracts of Curcuma and the applications of these extracts.

Plants of the Curcuma genus and, especially, Curcuma longa are tropical plants known from ancient times that belong to the Zingiberaceae family and they originated in Asia. Dried Curcuma rhizomes were already used in Asia as a spice and textile dye. They were also used in popular medicine to treat stomach ailments.

In the last few years, new pharmacological applications have appeared in the scientific literature: antibacterial activity, antiinflammatory activity, lipid peroxidation capacity, antihepatotoxic capacity and antitumorigenic capacity.

Curcuma extracts are yellow pigments called curcuminoids, basically comprised of: curcumin (diphenylmethane), demethoxycurcumin (hydroxycinnamoylphenylmethane) and bisdeoxycurcumin (dihydroxycinnamoylmethane).

The method of the present invention, for obtaining the apolar extract of Curcuma longa is characterized in that it comprises the following operations:

- 10 a) subjecting the previously cut, dried and powdered Curcuma rhizomes to a continuos extraction process for 48 hours, using an organic solvent in a rhizome:solvent ratio of 1:7 weight/volume, carrying out the process in a stationary system to prevent losses of the solvent, for which purpose the temperature of the extraction boiler is adjusted;
- b) filtering the extraction mixture resulting from step (a) and evaporating the solvent from the filtrate obtained at reduced pressure obtaining a characteristic brown colored oleoresin as a dry residue;
- 15 c) dissolving the oleoresin resulting from step (b) in hot ethyl acetate, in a proportion of 50% w/w and then letting the solution cool down to room temperature for about 24 hours. An orange-colored precipitate that is separated by filtration appears;
- d) drying the filtrate coming from the previous step at reduced pressure to obtain a second oleoresin darker than the previous one, whose curcuminoid content is from 20 to 25 %;
- 20 e) recrystallizing the resulting precipitate in ethyl acetate 50% (w/w) to obtain a solid with a purity in curcuminoids higher than 90%.

On its part, the method of the present invention for obtaining the polar extract of Curcuma longa is characterized in that it comprises the following operations:

- 25 a) subjecting the previously cut, dried and powdered Curcuma longa rhizomes to an extraction process with water, keeping them in maceration for about 24 hours at a temperature between 50 and 70° C;
- b) filtering the extraction mixture resulting from step (a) and evaporating the water from the filtrate this obtained at reduced pressure to obtain a residue that is a hygroscopic solid corresponding to the polar extract of Curcuma longa.

Likewise, the method of the present invention is carried out by supercritical extraction, for which purpose the Curcuma rhizome, previously cut, dried and powdered is extracted with ethanol/CO₂ and at a pressure of about 260 bar, to obtain the corresponding apolar extract with a purity in curcuminoids of 90%. The apolar extract thus obtained is identical to the one obtained by extraction with solvents according to what is shown by the spectroscopic and chromatographic data thereof. In the same way, but using water as a cosolvent, the polar extract, whose fine layer chromatography totally coincides with that of the extract obtained by maceration in water indicated above, is obtained.

Preferably, obtainment of the above mentioned apolar extract, by Supercritical extraction is carried out in the following conditions:

- 40 - Drying the cut Curcuma longa at a temperature of 50° C;
- Grinding the rhizome into particles between 0.-1 and 1 mm;
- Extracting the Curcuma longa rhizome using ethanol, modified with a small amount of CO₂, as a solvent, at 250 bars and 50° C;
- 45 - Extracting the ethanol present in the oleoresin with CO₂ at 280 bars and 50° C;

The apolar and polar extracts of Curcuma thus obtained have a capacity to catch free radicals, specifically, OH[•] hydroxyl and O₂[•] superoxide radicals as is shown hereinafter by the studies carried out by the inventors in this regard. Besides, said extracts, as it is also shown hereinafter, have a capacity to reduce the plasma levels of lipid peroxides in 50 human beings. Consequently, they have beneficial effects in pathologies related to cell oxidation such as arteriosclerosis and rheumatoid arthritis.

The present invention also includes compositions and preparations with a capacity to catch free radicals, which are effective against ageing processes, protection of genetic material and antimutagenic capacity. Likewise, it includes compositions and preparations with a capacity to reduce the plasma levels of lipid peroxides.

55 The cited compositions or preparations contain as active principles the polar or apolar extracts of Curcuma longa rhizome obtained by the method of the invention or mixtures of the same, along with pharmaceutical quality excipients suitable for oral administration.

These oral preparations can come in solid form (powder, tablets, dragees, capsules, etc.) or in liquid form (syrups or suspensions).

In accordance with what has been said above, the present inventors show as a protective agent or catcher of free radicals, aside from the apolar extract of *Curcuma longa*, which the curcuminoids are in, the aqueous or polar extract of *Curcuma longa* in which there is no curcumin nor curcuminoid. Besides, the studies carried out by the cited inventors, show that the aqueous extract of *Curcuma longa* is in itself a strong catcher of free radicals, especially superoxide (O_2^-).

The relative application to the reduction of the plasma levels of lipid peroxides is fundamentally based on the capacity to catch free radicals of the cited extracts.

Within the field of study of ageing, it is necessary to evaluate different variables that belong to the scopes of physiology, biochemistry and psychology. The studies directed towards preventive gerontology have lead to the creation of the profile of biological age and life style, that intend to be a tool that evaluates in the strictest and simplest possible way the most sensitive parameters regarding vital habits such as: Pharmacological treatment, tobacco, alcohol, exercise, sleep, perception of one's own health and stress.

Apart from the life style of the population in general, there are biochemical parameters such as total cholesterol, triglycerides, glucose, uric acid and HDL/cholesterol, which are considered important in the evaluation of the degree of ageing.

In this sense, due to the large amount of information that it creates, it is interesting to include in the profile of biological age, a biochemical parameter, that can be measured rather easily in peripheral blood: lipid peroxides, resulting from the oxidation by the free radicals derived from oxygen, on the polysaturated fatty acids that form part of the phospholipids of membranes.

This oxidative process frequently exceeds the homeostatic capacity of the cell and there may be an abnormal and uncontrolled oxidation process with massive destruction of the cell membrane. It is known that in homeostasis of peroxidation antioxidants such as betacarotenes and tocopherols take part. Therefore, it is logical to think that exogenous administration of an antioxidant, can be useful to reestablish this cellular balance.

The present inventors have studied the antioxidantizing capacity of pharmaceutical compositions comprised of polar and apolar extracts of *Curcuma longa*, for their action on the serum levels of malonaldehyde (MDA) in human beings, as an indicator of their blocking capacity of the action of free radicals of the cell membrane. The conclusions obtained from this study are the following:

1. *Curcuma longa* is a natural extract that reduces the plasma levels of lipid peroxides, which implies a protective mechanism of the cell membrane.
2. It has no toxicity at all: the intake of *Curcuma longa* for 105 days does not modify the liver and kidney blood tests.
3. The effect at the level of lipid peroxides is rapid: 45 days later a drop of the same was already detected, practically in all decades and in both sexes.
4. The inhibition of lipid peroxides and the catch of free radicals, basic properties of *Curcuma longa* are one of the grounds on which the theories of ageing are based.

BRIEF DESCRIPTION OF THE FIGURES

Figure 1: Values of the lipid peroxide levels for the males of the study.

Figure 2: Values of the lipid peroxide levels for the females of the study.

EMBODIMENTS OF THE INVENTION

The present invention is additionally illustrated by the following Examples, which do not have the intention of limiting its scope.

EXAMPLE 1

This example illustrates the obtainment of the apolar extract of Curcuma of the present invention.

50 13-14 kg. of dry ground Curcuma rhizome were subjected to continuous extraction using 70 liters of dichloromethane. The temperature of the boiler was adjusted until a stationary system was obtained.

Extraction was considered to be finished after 48 hours.

The extract was filtered and the solvent evaporated at reduced pressure (0.5 torr) until it was totally eliminated.

The oleoresin yield using this method varied between 12-15% based on the weight of the starting dry rhizome.

55 The oleoresin thus obtained was dissolved in hot ethyl acetate (50% by weight) and was left at room temperature for 24 hours, during the course of which an orange-colored precipitate (curcuminoids) that was separated by filtration, appeared little by little.

The filtrate was dried at reduced pressure until a second oleoresin darker than the previous one whose curcuminoid content varied between 20% and 25% was obtained. The precipitate was recrystallized in ethyl acetate (50% by weight) following the above mentioned steps, thus obtaining curcuminoids with a purity higher than 90%.

5 EXAMPLE 2

This example illustrates the obtainment of the polar extract of Curcuma of the present invention.

30 kg. of ground Curcuma longa rhizomes were extracted with 300 liters of water at 70° C in maceration for 24 hours. The solution was filtered through a filtering plate and the solvent was evaporated at reduced pressure (0.5 bar) 10 obtaining 240 gr. of a hygroscopic solid. Yield 8%.

EXAMPLE 3

This example reveals how the extraction process is optimized by using fluids in a supercritical state, studying the 15 following parameters:

- Thermal stability of curcuminoids
- Study of the extraction temperature
- Study of the feed pressure
- 20 - Study of the particle size
- Study of the humidity of the starting matrix
- Study of the flow of the supercritical fluid
- Study of the use of ethanol as a cosolvent
- Extraction by combined techniques

25 As of the experimental results, costs have been evaluated and the optimal method is the one that includes the following steps:

- Drying the cut Curcuma longa at a temperature of 50° C.
- Grinding the rhizome in particles between 0.1 and 1 mm.
- 30 - Extracting the Curcuma longa rhizome using ethanol, modified with a small amount of CO₂ as the solvent at 250 bars and 50° C.
- Extracting of the ethanol present in the oleoresin with CO₂ at 280 bars and 50° C.

The novelty of this techniques lies in the use of ethanol modified with CO₂ for extracting Curcuma longa and 35 on the other hand this method reduces costs for extracting curcumin in contrast to conventional techniques, extraction with solvents and/or supercritical extraction with carbon dioxide.

This extraction method increases the selectivity of the extraction of curcumin in contrast to other curcuminoids.

- Stability in curcumin
 - The curcumin present in the apolar fraction shows a thermal degradation in terms of time.
 - 40 The results indicate a loss of curcuminoids as the temperature rises, above all upon exceeding 50° C.
 - Initially, a greater degradation is produced which is stabilized as of 24-48 hours of exposure. Degradation is kept at 2% for temperatures lower than 50° C and at 4% at 80° C.
- Study of the extraction temperature
 - The extraction yield at 250 bar with a flow of 3 kg/h of CO₂ and a particle size between 0.1 and 1 mm at 35° C, 40° C, 50° C, 55° C has been studied and the best yields have been obtained at 55° C.
- Study of the feed pressure
 - The extraction yield has been studied at different temperatures (30° C, 40° C, 50° C) and at different pressures (100 bar, 150 bar, 200 bar, 250 bar) with a flow of 3 kg/h of CO₂ and a particle size between 0.1 and 1 mm. For all the temperatures studied, the maximum yield obtained has been at 250 bars of pressure.
- 50 - Study of the particle size
 - The extraction yield has been studied at 50° C, 250 bar and a flow of 3 kg/h of CO₂ for different particle sizes.
 - The maximum yield was obtained for particle sizes between 0.1 and 1 millimeter.
- Study of the moisture of the starting matrix
 - The extraction yield from different samples of Curcuma rhizomes with a different degree of humidity (80%, 55% 50%, 8%) has been studied. The highest yield of curcumin is obtained working at a humidity in the starting matrix of 8%, therefore to obtain the apolar fraction the extraction process is carried out with the dehydrated system.
- Study of the flow of supercritical fluid

It has been observed that as the flow of solvent reduces, the onset of diffusive effects is delayed, increasing the solubility effect and increasing the extraction yield. On the other hand, excessively small and/or large flows increase the extraction time.

- Extraction with ethanol and supercritical carbon dioxide

The curcuminoid yield has been studied by extracting Curcuma with different proportions of cosolvent (ethanol) in CO₂.

The highest yield of oleoresin was obtained by using ethanol 96% with 4% CO₂, obtaining a yield of oleoresin of 11.42% (with 43% of curcuminoids) which means 98% of the total amount of the oleoresin in the initial product and a yield of 100% of total curcuminoids.

- Extraction by combined techniques

From the supercritical extractions as of Curcuma longa oleoresins obtained by extraction with a solvent. It was observed that the extraction was very fast at the beginning, but it began to slow down with time by solidification of the oleoresin. This solid had a compact appearance and it was formed by very fine particles.

As the main component the extract had the solvent used in the extraction.

In these tests the existence of a large cosolvent effect was observed, since the extract obtained was introduced again in the equipment to see if it could be fractionated and it was observed that it was not very soluble in supercritical CO₂.

An oleoresin with ethanol and CO₂ 96-4% was attained at 250 bar and at 50° C and then this oleoresin was extracted at 280 bar, 50° C and using a flow of 3 kg/h of CO₂.

The obtained solid oleoresin showed a yield similar to the yield obtained by conventional methods but it increased in the selectivity of the extraction of the oleoresin in the extraction with ethanol in contrast to the extraction with organic solvents. The results are given hereinafter.

	Curcumin %	Demethoxy %	Bisdemethoxy %
Oleoresin obtained by conventional methods	34.50 %	29.50%	36.00%
Oleoresin obtained with EtOH	72.36%	17.76%	9.88%

- Cost estimate

From the above cited studies an estimate of costs has been made studying the different possible cases, calculating the cost per kg of curcumin. The different possibilities are:

a.- Starting with Curcuma longa and extracting the oleoresin with CO₂ in a supercritical state.

- 1.- Flow of 3 kg/h and apparent density of Curcuma of 0.413 kg/l. Possibility 1.1.
- 2.- Flow of 3 kg/h and apparent density of Curcuma of 0.573 kg/l. Possibility case 1.2.
- 3.- Flow of 1 kg/h and apparent density of Curcuma of 0.413 kg/l. Possibility 1.3.

b.- Using Curcuma longa as the raw material and extracting the oleoresin by a process in batches using a mixture of ethanol/carbon dioxide at a high pressure as the solvent. Possibility 2.

c.- Starting with the oleoresin extracted with organic solvents by conventional methods and extracting the solvents present with CO₂ in a supercritical state. In this way a solid resin more concentrated in curcumin is obtained. Possibility 3.

d.- Starting with an oleoresin extracted with ethanol/CO₂ at a high pressure and extracting the ethanol present with CO₂ in a supercritical state. In this way a solid oleoresin even more concentrated in curcumin than the previous one is obtained. Possibility 4.

e.- Starting with Curcuma longa and extracting the oleoresin with CO₂ SCX modified with ethanol.

- 1.- Proportion of ethanol in the solvent of 17%. Possibility 6.1.
- 2.- Proportion of ethanol in the solvent of 34%. Possibility 6.2.

Operation

In order to carry out the cost estimate, an effectiveness of 90% of the total of the operation has been considered.

Description Possibility	Operating conditions	Us/Ma	\$	Operation time	Extracting volume	Volume time 1	Total cost
P(bar)	T(°C)	(Kg/h)/Kg	(Kg/Kg)	(h)	(L)	(L.h)	(PTA/Kg)
Possibility 1.1	250	50	24,61	38	1,54	24213	37288
Possibility 1.2	250	50	17,74	60	3,38	17452	58988
Possibility 1.3	250	50	7,41	13	1,76	24213	42615
Possibility 2	250	50		2,16	1,47	5381	7910
Possibility 3	280	50	26,95	66	2,45	482	1181
Possibility 4	280	50	26,83	33	1,23	260	320
Possibility 5	1	80	6,10	2,53	1,50	5906	8859
Possibility 6.1	250	50	25,10	60	2,39	5381	12861
Possibility 6.2	250	50	23,73	20	0,89	5381	4520
							2222

It is observed that the most favorable possibility is possibility number 4 wherein the Curcuma longa rhizome is extracted with ethanol under pressure and then the oleoresin is purified by extraction with CO₂.

Installation

In order to carry out the obtainment of curcumin from its oleoresin, two extractors and two separators, provided with heating jackets, are needed.

The equipment must be equipped with a pump with internal cooling, capable of providing pressures of 300 bar. Besides, a cooler to liqueate the CO₂ that comes out of the second separator is needed in order to be able to thus recirculate the CO₂ lowering operating costs.

The fact of having two extractors is so that a semicontinuous extraction process can be carried out, since during the inoperative times of loading and unloading and pressurizing the unit, the process can continue to operate with the other extractor.

The plant must be equipped with a system to control the pressure and temperature in the extractor and separator and the flow and temperature of the cooler. Besides, a pressure and temperature alarm system must be installed in the extractors, separators and heating jackets, as well as in the cooler in order to prevent any accident.

15 **EXAMPLE 4**

This example illustrates a composition in accordance with the present invention, to be administered orally, in the form of tablets comprised of:

20	- Curcuminoids	15 mg.
	- Aqueous Curcuma extract	175 mg.
25	- Avicel pH 102	489 mg.
	- Starch	45 mg.
	- Magnesium stearate	8 mg.
	- Primogel	3 mg.
30	- EM compress	15 mg.

35 **EXAMPLE 5**

This example illustrates a liquid composition, in accordance with the present invention, to be administered orally, as an emulsion, comprised of:

40	- Curcuminoids	20 mg.
	- Aqueous Curcuma extract	200 mg.

45 along with variable amounts of sweeteners (for example, saccharose or fructose) and flavourings (for example, mint or strawberry essence) and of vegetable oils in a sufficient amount for 100 ml. of emulsion.

The following examples corresponds to the studies carried out on the extracts of the invention, directed towards determining and evaluating the capacity thereof to catch free radicals, as well as the capacity thereof to reduce lipid peroxides.

50 **EXAMPLE 6**Study of the capacity to catch the superoxide radical (O₂^{-·}) by the pyrogallol method

55 Self-oxidation of pyrogallol after an induction period of 20 seconds and for 2 minutes, time in which the increase of absorbency is linear at 420 nm, has been studied. The inhibition of the self-oxidation of pyrogallol by curcuma extracts is used to determine the capacity to catch the O₂^{-·} radical.

The experiment is carried out at 25° C. The pyrogallol is carried out hydrochloric acid 10 mM. The reaction is started when an air saturated Tris-cacodylic acid buffer, with a pH of 8.2 that contains 1 nM of DTPA pyrogalol, is

added. The DTPA is used as a chelating agent to eliminate possible interferences of ions such as iron, copper or manganese.

For apolar extracts of Curcuma a inhibition percentage of $40.92\% \pm 0.002$ ($p < 0.01$) at a concentration of 16.129 $\mu\text{g/mL}$ in curcuminoids was found, while the DSO inhibition is of 95%.

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EXAMPLE 7

Study of the capacity to catch the hydroxyl radical (OH^\cdot) by the DMSO method

10 Capacity to catch the hydroxyl radical by the DMSO test.

The capacity to catch free hydroxyl radicals (OH^\cdot) by measurement of the formaldehyde generated during the oxidation of the DMSO; a strong catcher of hydroxyl radicals by the ascorbic acid/ Fe^{+3} , has been studied. Oxidation is set off by the ascorbate and it is catalyzed by the Fe^{+3} at 37° C. The reaction mixture is formed with EDTA (0.1 mM, Fe^{+++} EDTA complex (1:2) (167 μM) DMSO (33 mM), problem extract and phosphate buffer 50 mM pH 7.4 in a final volume of 3 mL.

15 The ascorbic acid (2 mM) is added and after 30 minutes of incubation at 37° C, the reaction is stopped with 1 mL of trifluoracetic acid 17.5% (w/v), and it is measured in an aliquot of 1.5 mL by spectrophotometry.

20 The competence between the problem extract and the DMSO by the hydroxyl radical (OH^\cdot) generated coming from the ascorbic/iron system is used to estimate the catching activity of the hydroxyl radical OH^\cdot of the problem substance, expressing it as the percentage of inhibition of the production of formaldehyde.

The protocol used is the following:

EDTA	B μL	C μL	B μL	P μL
Fe ⁺⁺⁺ EDTA	250	250	250	250
DMSO	250	250	250	250
VEHICLE	500	500	---	---
EXTRACT	---	---	500	500
PHOSPHATE	250	---	---	---
BUFFER				
ASCORBATE	---	250	250	250
Tc: Control target				
C: Control				
Tp: Problem target				
P: Problem				

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The formaldehyde produced is measured in the previous action by addition of 2 mL of a reactive mixture of ammonium acetate and acetylacetone in a phosphate buffer 50 mM pH 7.4, on an aliquot of 1.5 mL. The mixture is incubated at 58° C for 5 minutes and then the samples are submerged in an ice bath and the 3,5-diacetyl-1,4dihydrolutin formed at 412 nm is measured.

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The results obtained were: for the apolar extract curcuminoid mixture it was $95.13 \pm 2.46\%$ ($P < 0.01$, $n=6$) the concentration of the sample being 16.129 $\mu\text{g/mL}$.

EXAMPLE 8

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Study of the capacity to catch the superoxide radical (O_2^\cdot) by the NADH-phenazinemethosulfate- O_2 -nitrotetrazolium blue (NBT)

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The capacity to catch free superoxide radicals by inhibition of the formation of diformaran, a highly colored compound that has a maximum absorption at 560 nm is studied. The reaction mixture is comprised of phosphate buffer 19 mM pH 7.4 NADH (90 μM), NBT (43 μM), extract to be tested (vehicle for the target), pH 5 (2.7 μM), all in a volume of 3.1 mL. The reduction reaction of the NBT is started by adding PMS and it is followed spectrophotometrically, by measuring the absorbency at 560 nm and at 20° C for 2 minutes to calculate the increase of absorbency by ($\Delta\text{A}/\text{min}$). The following scheme shows the protocol used:

	Bc(µL)	C (µL)	Bp (µL)	P(µL)
5	PHOSPHATE BUFFER	250	250	250
	NADH	100	100	100
	NBT	100	100	100
10	EXTRACT	---	---	100
	VEHICLE	100	100	---
	PMS	---	100	---
15	Tc: Control target C: Control Tp: Problem target P: Problem			

20 The percentages of inhibition are obtained for the polar extract of Smilax Lundeli for 5 samples with a concentration of 500 µg/mL, which is of $99.6 \pm 0.1\%$ for $p < 0.1$.

EXAMPLE 9

25 Study of the capacity to catch the superoxide radical (O_2^-) by the Xanthine-Xanthine method

30 The capacity to catch the superoxide radical of the polar fraction of *Curcuma longa* by the xanthine-xanthine oxidase method has been studied and there has been an inhibition of 77.95% for a final concentration of 1.25 µg/mL in curcuminoids. It has been verified that the extract does not inhibit the enzyme, which indicates that this extract is a strong catcher of superoxide radicals.

EXAMPLE 10

Study of the effects of the Curcuma longa extracts on the levels of lipid peroxides in human blood

35 This example intends to show the action, on the plasma levels of lipid peroxides, of a pharmaceutical composition comprised of polar and apolar extracts of *Curcuma longa*, when it is administered orally, in the form of two tablets daily containing 7 to 14 mg. of curcumin per tablet and from 150 to 175 mg. of aqueous extract of *Curcuma longa*, to a group of healthy subjects of both sexes for a period of two and a half months.

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COMPOSITION OF TABLETS OF THE PRESENT EXAMPLE

		mg/tablet
5	Apolar extract of <i>Curcuma longa</i>	60*
10	Polar extract of <i>Curcuma longa</i>	175
15	Avicel pH 102	489
	Cornstarch	45
	Magnesium stearate	8
	Primogel	3
	EM Compress	15

* Equivalent to 12.5 mg. of Curcuminoids

20

METHOD

A population of 19 males and 12 females of ages between 27 and 74 years old has been studied, just as it is indicated in the following Table 1:

TABLE 1

Number of subjects studied distributed by ages and sex	
Men	
Ages	Frequency
25-30	3
31-40	3
41-50	4
51-60	7
60-70	2
Women	
Ages	Frequency
30-40	3
41-50	4
51-75	6

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Peripheral blood was taken from these subjects for the determination of a complete hemogram, of the parameters of evaluation of liver and kidney function; likewise, the level of lipids and lipid peroxides in serum was determined.

After knowing the results, treatment consisting of two tablets daily of *Curcuma longa* with the following protocol was started: second taking of blood fifteen days after administration, determining the above cited parameters. The protocol follow-up was maintained, repeating the blood tests forty-five and seventy-five days after the study started. After this protocol was completed, a 30-day period rest period during which no tablets were administered took place and afterwards the above cited parameters were determined again.

ANALYTICAL METHODS

The hemogram was carried out in a Technicon H-1 analyzer (USA).

5 The biochemical parameters were carried out following standardized Boehringer-Mannheim reagents (Germany), with a Hitachi 717 self-analyzer (Japan).

10 The lipid peroxides were determined with malonaldehyde (MDA) by the Ohkama, Ohishi and Yagi method (Analytical Biochem. 95 351-358 (1979)), modified for serum.

RESULTS

10 During the protocol the patients did not report any of the following signs: dysphagia, pyrosis, nausea, vomiting, diarrhea or constipation.

15 No alterations either in the hemogram or in the biochemical parameters were observed in the entire population studied, without any distinction of sex or age. It was observed that the enzymes marking liver function, (GOT, GPT, GGT, alkaline phosphatase) as indicators of toxicity of the product and total direct and indirect bilirubin were not affected during the entire process, as one can see in the following Table 3. Said Table 3 shows the initial and final values of GOT, GPT and alkaline phosphatase distributed by age groups in terms of sex. The average and standard deviation are indicated. The units in said table are the following:

20 (a) Initial Time = Time 0
(b) Final Time = 105 days from the beginning
(c) Reference values for the indicated analytical method:

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TABLE 3:

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GOT								GPT								
Men	VR (c)	Initial t (a)	Final time (b)	VR	Initial time	Final time		Men	VR (c)	Initial t (a)	Final time (b)	VR	Initial time	Final time		
Age		Average	St. dev.	Average	St. dev.	Average	St. dev.	Age		Average	St. dev.	Average	St. dev.	Average	St. dev.	
25-30	0-30	21,3	3,1	22,3	3,1	0-40	19,0	5,2	18,3	3,5						
31-40	0-30	24,3	10,4	20,7	4,0	0-40	23,7	6,6	19,3	4,3						
41-50	0-30	23,0	5,1	26,0	1,7	0-40	30,8	16,9	33,0	11,3						
51-60	0-30	20,0	8,0	23,0	7,7	0-40	21,0	10,7	21,8	4,0						
60-70	0-30	21,0	2,8	23,0	4,2	0-40	20,0	5,7	17,0	4,2						
Women																
Age	0-30	Average	St. dev.	Average	St. dev.	0-40	Average	St. dev.	Average	St. dev.						
30-40	0-30	21,3	11,0	27,7	18,5	0-40	27,0	26,9	32,3	34,4						
41-50	0-30	15,0	2,2	15,5	0,7	0-40	11,0	2,6	10,0	0,0						
51-75	0-30	16,7	3,0	20,0	2,8	0-40	15,7	3,0	12,0	5,7						

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GGT				Alkaline phosphatase			
Men	VR	Initial t	Final t	VR	Initial t.	Final t.	
Age		Average	St. dev.	Average	St. dev.	Average	St. dev.
25-30	11-50	18,3	1,5	9,0	13,9	98-270	179,0
31-40	11-50	25,0	11,2	25,7	18,1	98-270	129,3
41-50	11-50	25,3	10,5	28,3	33,5	98-270	129,3
51-60	11-50	32,2	13,0	96,5	48,1	98-270	160,8
60-70	11-50	20,5	2,5	79,7	19,8	98-270	111,0
Women							
Age	11-50	Average	St. dev.	Average	St. dev.	98-270	Average
30-40	11-50	18,7	4,0	19,0	13,9	98-270	135,0
41-50	11-50	12,3	5,0	8,5	29,6	98-270	123,3
51-75	11-50	12,8	5,2	7,5	61,8	98-270	160,8

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As is shown in Figure 1, after 15 days of treatment, a reduction around 8 % of the level of lipid peroxides was observed in males, while 45 days after treatment started, the reduction reached an average value of 34%, in such a way that the variation with regard to the situation after 15 days of all of the males subjected to the study will be 59%. In females, an average increase in the level of lipid peroxides (26%) was observed after 15 days (Fig. 2); however, after 45 days, the reduction of the level of peroxides is more evident (it drops an average of 11%), affecting practically all of the subjects; these results imply an average reduction after 45 days with regard to the situation of women subjected to the study after 15 days, of 80%.

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Claims

- Method for obtaining apolar and polar extracts of Curcuma, characterized in that (I) to obtain the apolar extract of Curcuma longa the following operations are carried out:

(a) subjecting previously cut, dried and powdered Curcuma rhizomes to a continuos extraction process for 48 hours, using an organic solvent in a rhizome:solvent ratio of 1:7 weight/volume, carrying out the process in a stationary system by means of adjusting the temperature;

5 (b) filtering the extraction mixture resulting from step (a) and evaporating the solvent from the filtrate obtained at reduced pressure obtaining a characteristic brown colored oleoresin as a dry residue;

(c) dissolving the oleoresin resulting from step (b) in hot ethyl acetate, in a proportion of about 50% w/w and then letting the solution cool to room temperature for about 24 hours. An orange-colored precipitate that is separated by filtration appears;

(d) drying at reduced pressure, the filtrate coming from the previous step to obtain a second oleoresin darker than the previous one, whose curcuminoid content varies between 20 and 25%;

10 recrystallizing the resulting precipitate in ethyl acetate about 50% (w/w) to obtain a solid having a purity in curcuminoids higher than 90%;

and in that (II) to obtain the polar extract of Curcuma longa the following operations are carried out:

15 (a) subjecting the previously cut, dried and powdered Curcuma longa rhizomes to an extraction process with water, keeping them in maceration for about 24 hours at a temperature between 50 and 70° C;

(b) filtering the extraction mixture resulting from step (a) and evaporating the water from the filtrate thus obtained at reduced pressure to obtain a residue that is a hygroscopic solid corresponding to the polar extract of Curcuma longa;

20 and in that (III) it is also possible to carry out the extractions by the supercritical extraction technique using ethanol/CO₂ and at a pressure of about 260 bar to obtain the corresponding apolar extract having a purity in curcuminoids of 90%; or using water as a cosolvent in the same conditions to obtain the polar extract.

25 2. Method according to claim 1, characterized in that the supercritical extraction of the apolar extract is carried out with Curcuma longa rhizome dried at about 50° C and ground to a particle size of 0.1-1 mm, carrying out the extraction with ethanol, modified with a small amount of CO₂ as a solvent at 250 bars and 50° C.

30 3. Application of the apolar and polar extracts obtained by the method of claim 1, to manufacture compositions and preparations useful as agents to catch radicals and antiageing agents.

4. Application according to claim 3, of the cited polar extract to manufacture compositions that catch superoxide radicals.

35 5. Application of the apolar and polar extracts obtained by the method of claim 1, to manufacture compositions and preparations useful as reducing agents of the plasma levels of lipid peroxides in human beings.

6. Application of the apolar and polar extracts obtained by the method of claim 1, to manufacture compositions and preparations with beneficial effects in pathologies related to cell oxidation.

40 7. Application according to claim 6, characterized in that said pathologies are arteriosclerosis and rheumatoid arthritis.

8. Application according to claims 3 to 7 characterized in that the cited composition can be administered orally and may be a solid or liquid.

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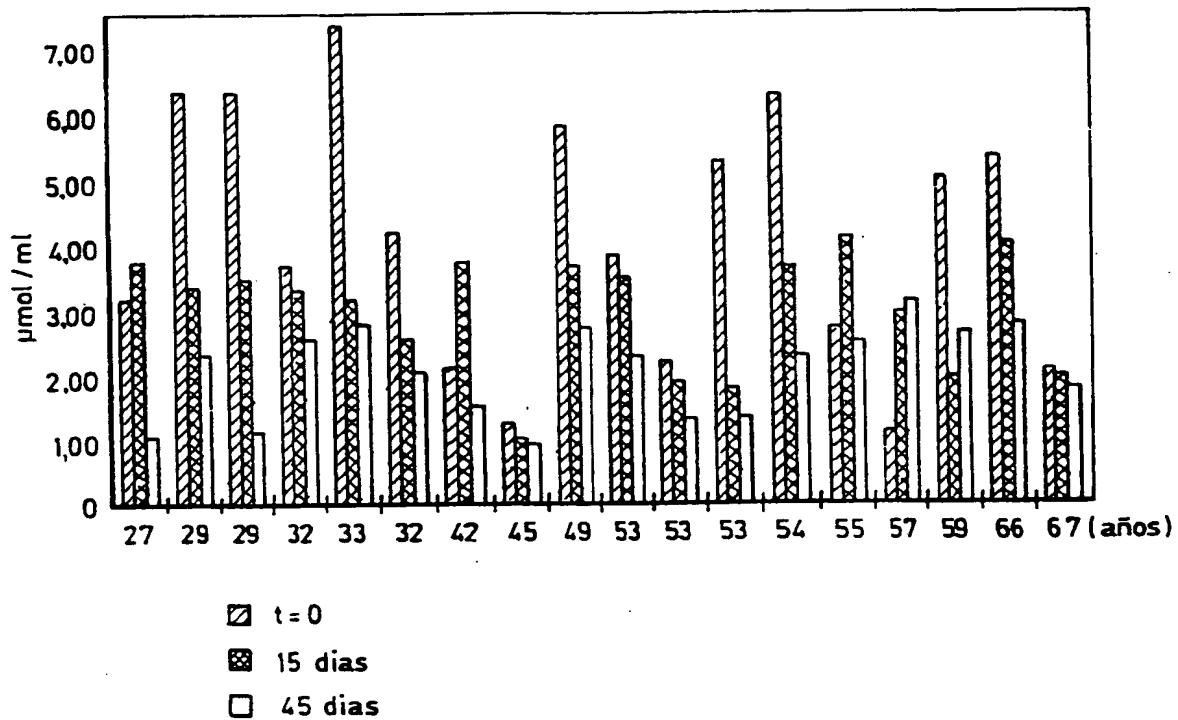


FIG.1

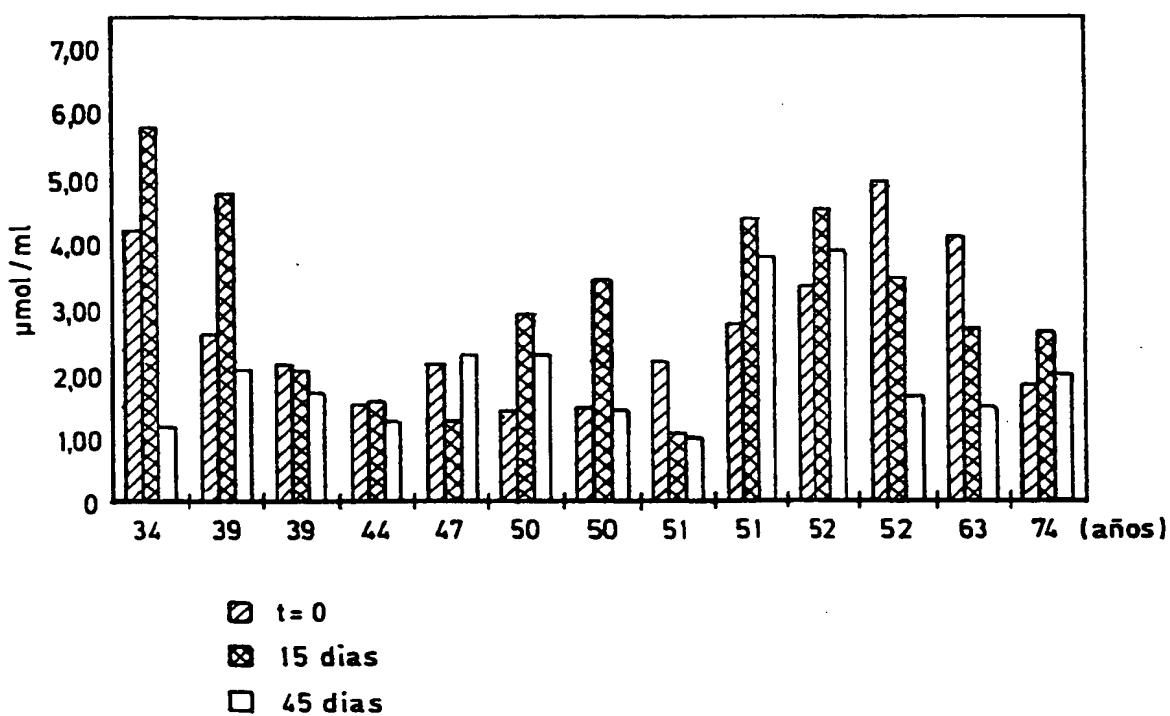


FIG. 2

INTERNATIONAL SEARCH REPORT

International application No.

PCT/ES 95/00097

A. CLASSIFICATION OF SUBJECT MATTER

IPC⁶: A61K 35778

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC⁶: A61K 35/78

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

CIBEPAT, CD-CIBEPAT, EPODOC, WPIL, CAS, BIOSIS, MEDLINE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US 5266344 A (NIMURA et al.) 30.11.93, columns 1, 2, 6, 7; example 3.	1,3,4,5,6
Y	Base de datos WPIL en Questel, semana 9407, Londres: Derwent Publications Ltd., AN 94-053939, Class B05, JP 6009479 A (OKINAWA KEN KINOSEI SHOKUHIN), abstract.	1
Y	FR 761734 A (A. BOERRIGTER) 26.03.34, page 2, lines 11-44.	1
Y	FR 2655054 A (PACIFIC CHEMICAL CO. LTD.) 31.05.95, pages 1, 2, 3, 6; claim 1.	3,4,6
Y	SRIVASTANA, K.C., MUSTAFA T. Pharmacological effets of spices: eicosanoid modulating activities and their significance in human health. Biomedical Reviews, 1993, vol.2, pages 15-29.	5,6,7,8

 Further documents are listed in the continuation of Box C. See patent family annex.

* Special categories of cited documents:

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"T" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and so is conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search

5 October 1995 (5.10.95)

Date of mailing of the international search report

(2.11.95)

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INTERNATIONAL SEARCH REPORT

International application No.
PCT/ES 95/00097

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	SRINIVAS, L., SHALINI V.K. SHYLAJA M., Turmerin: A water Soluble Antioxidant Peptide from Turmeric (Curcuma longa). Archives of Biochemistry and Biophysics. February 1992, Vol. 292, N°2, pages 617-623, abstract.	5,6
Y	CHANDRA D., GUPTA S.S. Anti-inflammatory and Anti-arthritis Activity of Volatile oil of Curcuma longa (Haldi). Indian J. Med. Res., January 1972, vol.60, n°1, pages 138-142	7,8
Y	SHALINI V.K., SRINIVAS L. Lipid peroxide induced DNA damage: protection by turmeric (Curcuma longa). Molecular and Cellular Biochemistry, 1987, vol.77, pages 3-10.	5
A	DE 2924345 A (A. NATTERMANN AND CIE GMBH) 08.01.81, all the document.	1
A	EP 0440885 A (PT. DARIA-VARIA LABORATORIA) 14.08.91, page 3, lines 31-37, examples 1,2.	1,8
A	US 4842859 A (LIU) 27.06.89, column 2, example 2.	1.5,7
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